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EFFECTS OF BIOSYNTHETIC HUMAN EPIDERMAL
GROWTH FACTOR ON WOUND HEALING

Final Report

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In conducting the research described in this report, the investigator
adhered to the "Guide for the Care and Use of Laboratory Animals,"
prepared by the Committee on Care and Use of Laboratory Animals of the
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STATEMENT OF PROBLEM UNDER STUDY

Much of the mortality, morbidity, and cost of caring for patients with major injuries such as burns or extensive surgery is closely related to the length of time required for their injuries to heal. At present, the ability of physicians to accelerate healing of mid-dermal injuries and incisions is severely limited and relies primarily on preventing infections and providing proper nutritional support. Thus, an agent that would accelerate wound healing would be highly desirable. Our hypothesis is that locally produced peptide growth factors regulate wound healing by autocrine/paracrine mechanisms. This implies that retarded healing is a result of inadequate production of growth promoting factors and their receptors (or the over production of growth inhibiting factors and their receptors). The major goals of this contract are to determine if treatment of wounds with biosynthetic peptide growth factors wounds can significantly accelerate healing of mid-dermal injuries, surgical incisions and tympanic membrane perforations.

BACKGROUND AND REVIEW OF APPROPRIATE LITERATURE AND EARLIER REPORT

Epidermal Regeneration. Patients with extensive second and third degree burns are at high risk for developing life-threatening infections and other complications until the epidermal layer of their skin has regenerated. The standard of treatment for full-thickness burns is tangential excision and autographing with split-thickness skin. Cadaver skin, pig skin, or placenta are all useful for temporary coverage of full-thickness injuries until adequate autographs can be obtained. But in severely burned patients, suitable donor sites are often the limiting factor in covering patient's burns. Substitutes for autologous skin graphs such as sheets of cultured epidermal keratinocytes or synthetic dermis containing fibroblasts from the patient have shown promise but are not yet a reality and require extensive technical support facilities. Thus, there is a need for a simple agent that would accelerate the rate of epidermal regeneration of mid-dermal injuries such as second degree burns or donor sites.

Previous reports indicated that epidermal growth factor (EGF), a small polypeptide growth factor found in human urine and blood, is a powerful mitogen for most ectodermal and mesodermal cells (1), and that EGF stimulated mitosis of keratinocytes in culture (2). Several early studies had reported that topical applications of EGF applied as a mist in saline failed to stimulate healing of epidermal injuries. Greaves (3) reported that brief (5 min) exposure of suction-induced epidermal wounds to EGF in saline solution once daily failed to accelerate wound closure. Arturson (4) also failed to find enhanced healing of mid-dermal thermal burns in rats treated once daily for 20 seconds with EGF in saline. Thorton et al. (5) applied EGF once daily as a mist to mid-dermal burns on rats and did not observe an increase in rate of healing. Franklin and Lynch (6), however reported a qualitative improvement in closure of full-thickness wounds in rabbit ears when treated with EGF in an ointment. We speculated that the reason for the failures of previous studies to detect an acceleration of epidermal regeneration was due to their lack of understanding of the mechanism of action of EGF on target cells which requires prolonged exposure to EGF to induce mitosis (7,8).

Surgical Incisions. Healing of surgical or traumatic incisions is a major factor in the time and cost required for total rehabilitation of patients. At present, no pharmacological agent is available clinically to accelerate healing of incisions.

Experiments performed with wound chambers in animals suggested that repeated injections of EGF or other peptide growth factors increased parameters associated with tensile strength such as content of collagen and DNA. Buckley et al. (9) implanted polyvinyl sponge disks subcutaneously beneath the ventral panniculus carnosus in rats and measured collagen content 20 days later. Daily injections of EGF into the sponges increased collagen content 26% compared to saline injection. Since the EGF rapidly disappeared from the injection site (only 10% remained after 4 hours), EGF was formulated in slow release pellets which were placed in the implanted sponges. At day 7, sponges receiving 10 μg of EGF released per day contained 41% increase in collagen content and pronounced increase in cellularity (DNA) and vascularity compared to saline control sponges. Similar results were reported by Laato et al. (10) following daily injections of EGF into cylindrical hollow sponges implanted subcutaneously on backs of rats, and by Grotendorst et al. (11) with collagen filled chambers containing EGF or platelet derived growth factors (PDGF).

RATIONALE USED IN CURRENT STUDY

Epidermal Regeneration. Based on the in vitro and in vivo data discussed above, we hypothesized that EGF could stimulate epidermal healing and cutaneous healing if applied in appropriate formulations. The model we chose for testing epidermal regeneration was mid-dermal thermal burns and split-thickness donor site injuries in pig. This animal model offered several distinct advantages over rats or guinea pigs. Pigs are large enough to sustain numerous test burns on the same animal without impairing the general health of the animal which allows several tests to be performed on the same animal. Thus, more direct comparisons could be made between experimental and control burns by minimizing variability in healing rates between different animals. Also, pig skin is very similar in structure and healing properties to human skin and pigs have been used extensively in burn research. Using this animal system we first tested EGF in different formulations including saline, lanolin, and a commercially available antibiotic cream, Silvadene. Both split-thickness incisions and mid-dermal thermal burns were utilized.

After we demonstrated that EGF significantly stimulated the rate of epidermal regeneration in mid-dermal injuries in pigs (12), we next evaluated other biosynthetic peptide growth factors which might have activity on keratinocytes using the same pig epidermal regeneration model. The rationale for selecting some of these factors was that they were structurally related to EGF (transforming growth factor alpha (TGF-α), vaccinia growth factor (VGF), and a synthetic hybrid molecule composed of the N-terminal half of TGF-α and the C-terminal half of VGF. Other factors were selected because they were general mitogens that might stimulate or inhibit mitosis of epidermal keratinocytes. These included basic fibroblast growth factor (FGF), insulin-like growth factor I (IGF-I), and transforming growth factor beta (TGF-β) (13).

In addition, we also performed a double blind clinical trial using EGF on paired donor sites. The rationale for selecting donor sites for the clinical trial was that donor sites provided uniform mid-dermal injuries in contrast to variable injuries produced by accidents. Second, donor sites could be selected on patients which were not likely to have impaired wound healing such as diabetics or patients receiving steroids or chemotherapy. Third, paired donor sites allowed for direct comparison of the rate of healing between EGF-treated and vehicle-treated injuries which would control for differences in natural healing rates between different patients.

Surgical Incisions. To study healing of surgical incisions, we used interscapular incisions of rats. This animal model offered the advantages of low cost per animal and the ability to produce an incision long enough to obtain multiple samples from each incision for tensile strength measurements. Also, rats have been used extensively for studies of incisional healing. We first tested EGF in different vehicles including saline, single lamellar liposomes, multilamellar liposomes, and viscous hydrogel solutions. Tensile strength measurements and histology were performed.

We also tested another growth factor, TGF-β, which had been reported to stimulate collagen production by fibroblasts in vitro and because TGF-β might not need prolonged exposure time to induce biological responses (14).

Tympanic Membrane Formations. We also conducted preliminary studies on the effects of EGF on healing of tympanic membrane (TM) perforations. The rationale for these studies is that the histological structure of TM is very similar to that of skin with a stratified squamous epithelium covering a stromal layer of fibroblasts and an inner layer of mucoepithelium. Thus, cells of the TM may be targets for EGF action. Perforations to TM can be caused by overpressures produced by explosions, and until the perforations heal, hearing is compromised and the patient is at greatly increased risk for infections in the inner ear which can lead to permanent hearing loss. No animal model existed for chronic, nonhealing perforations of TM, so we developed a model to study healing of performations in TM of cats.

EXPERIMENTAL METHODS

Epidermal Regeneration. Experiments were conducted as described by Brown et al. (12). Briefly, split-thickness donor sites 18/1000 inch were made by a dermatome on the dorsal thorax of pigs (40 to 80 pounds). Twice a day, wounds were treated with vehicles only, vehicles containing growth factors or left untreated. At indicated times post injury, the injuries were photographed and the percentage of wound area judged to be re-epithelialized on enlarged prints was measured by computerized planimetry. Alternatively, split-thickness donor sites including 5 mm of surround normal skin were completely excised into the deep dermis and epidermal and dermal layers of the specimens were separated after incubation in trypsin solution overnight. Wounds were considered healed when no defect was present in the epidermal layer and not healed if any defect remained. Results were analyzed by chi-squared test for statistical significance.

Mid-dermal thermal injuries were made by contact for 10 seconds with a brass template (3x5 cm, 430 g) heated to 70°C in a constant-temperature water bath to the depiliated dorsal skin of pigs (50 to 80 pounds). After removing the blister, burns were treated twice a day with peptide growth factors in vehicle, vehicle alone, or untreated (12). At appropriate times after injury (approximately 7 days), the fibrinous coagulum was removed and burns were photographed and biopsy specimens taken. The percentage of the original burn area that appeared to have regenerated epidermis was calculated by computerized planimetry of enlarged photographs. Results were analyzed by t-test for statistical significance.

Clinical Trial of EGF Treatment of Donor Sites. Patients who required split-thickness grafting for various reasons were enrolled in a prospective, randomized, double-blind trial. Each patient had two donor sites of 5 cm x 15 cm created with a Padgett dermatome set at a depth of 12/1000 inch. Wounds were

treated topically twice daily with 0.5 ml/cm^2 of either Silvadene cream containing 10 μg hEGF per ml or Silvadene alone. Wounds were photographed daily and prints were analyzed by computer planimetry to determine the percentage of epithelialization of each wound. On day 5 post injury, 3 mm punch biopsies were obtained from each wound for histological analysis. Results were analyzed by paired t-test for statistical significance.

Surgical Incision Healing. Experiments were conducted as described by Mustoe et al. (15). Briefly, adult male Sprague Dawley rats were anesthetized with Ketamine and Rompum and a single incision 5 cm long was made in the dorsal midline at the caudal portion of the back of each rat. Test solutions were placed in the tract of the incision, and incisions were closed with five evenly placed interrupted horizontal mattress sutures of 4-0 nylon. Rats were housed in individual cages with unrestricted feed and water. At intervals after surgery, rats were sacrificed and incisions together with surrounding normal skin were dissected to the panniculus carnosum, then placed on ice-cooled dishes until tested for tensile strength. For each incision, three strips of tissue were cut perpendicular to the original incision, and measured for tensile strength using an automatic tensometer (Unite-O-Matic Instruments). The values of the three strips were averaged to determine the tensile strength for each incision. For each experimental condition, the tensile strength was calculated by averaging the tensile strength for all incisions in that test group. Specimens for histology were placed in 10% neutral buffered formalin, processed through parafin, sectioned, and stained with hematoxyline and eosine.

Multilamellar liposomes containing EGF were prepared using a previously described procedure (16) by adding EGF to a glass test containing lecithin which had been plated to the walls under a stream of nitrogen. One ml of phosphate buffered saline (PBS) containing 1 mg of EGF was added and immediately vortexed and sonicated, then the liposomes were separated from the solution by centrifugation at 20,000 x g for 20 min or by gel filtration through a 25 cm column of Agarose 4B. The amount of EGF entrapped in liposomes was calculated by measuring the amount of a trace ^{125}I-EGF present in the purified liposomes.

Single lamellar liposomes also were prepared as described previously (16). Briefly, a mixture of lipids containing lecithin, phosphatidylglycerol, and cholesterol were added to a round bottom flask, 5 ml of ether added and dried under a nitrogen stream. Five ml of ether was added followed by 1 ml of EGF (1 mg/ml) in PBS then immediately sonicated. The organic phase was removed by rotary evaporation and the single lamellar liposomes were formed, then purified by gel filtration or centrifugation as before.

To measure the retention of EGF in incisions, multilamellar and single lamellar liposomes, as well as a solution of EGF in PBS, were prepared as above with a trace amount of ^{125}I-EGF. Incisions were made as before and a known amount of EGF was placed in the incision tract then the incision was closed with sutures. At the indicated intervals, the incisions and surrounding skin were excised and ^{125}I-EGF measured by scintillation counting.

Tympanic Membrane Perforation Model. Cats underwent bilateral metoplasties and bilateral, total perforations TMs. Ears were treated with EGF in various formulations including saline or hydroxyproplymethyl cellulose. At the indicated times after injury, TMs were fixed in situ with formaldehyde, dissected from the bony annulus, photographed and the area of remaining perforations measured by planimetry. Results were analyzed by paired t-test.

RESULTS

Epidermal Regeneration. As shown in Figure 1, 50% of the split-thickness incisions treated with EGF in lanolin or in Silvadene were healed after 2 days of treatment, whereas vehicle-treated or untreated groups required greater than 4 days for 50% of the wounds to heal (p<0.05, chi-squared analysis). Histological evaluation of healed split-thickness wounds were similar in appearance for all three group wounds with a 10 to 12 cell layers of stratified epithelium characteristic of normal pig skin and no evidence of metaplastic transformation.

EGF-treatment of mid-dermal thermal burns significantly (p<0.01, Tukey's analysis of variance) increased the area of regenerated epidermis at 7 days after injury compared to vehicle-treated burns or untreated burns (Table 1). Biopsy specimens taken 7 days after burn injury confirmed complete epidermal regeneration of EGF-treated burns with stratification of cell layers. In contrast, very little epidermal regeneration was observed in vehicle-treated or untreated burns. Specimens taken 35 days after burn injury had totally normal histology of the epidermis and dermis.

Response of mid-dermal burns to varying doses of EGF is shown in Figure 2. All burns treated with EGF (0.1, 1, and 10 μg/ml) were significantly more healed than untreated burns, but only burns treated with EGF at 10 μg/ml were significantly more healed than vehicle-treated burns (p<0.05, analysis of variance).

Treatment of mid-dermal burns with TGF-α or VGF produced results similar to those observed with EGF-treatment. As shown in Table 2, treatment with TGF-α or VGF resulted in substantially more area re-epithelialized than untreated or vehicle-treated burns. Also, low levels of TGF-α (0.1 μg/ml) appear to be more effective than low levels of EGF in stimulating epidermal regeneration (Figure 3). Similar results were obtained with the hybrid molecule of TGF-α/VGF.

Treatment of split-thickness donor sites with TGF-β or combinations of EGF, IGF-I, and FGF produced the results shown in Figure 4. Treatment with TGF-β appeared to reduce the rate of epidermal regeneration.

Clinical Trial With EGF On Donor Sites. Twelve patients were enrolled in the study at Emory University. As shown in Table 2, donor sites treated with EGF healed at a significantly (p<0.05, paired t-test) faster rate than paired donor sites treated with vehicle. Morphometric analysis of histological specimens prepared from the punch biopsies also revealed substantially more epithelium present on the EGF-treated sites compared to vehicle-treated sites.

Cutaneous Incision Healing. Treatment of incisions at the time of suturing with EGF in saline failed to produce an increase in tensile strength even at high levels of EGF (100 μg). In contrast, when repetitive doses of EGF were given three times a day for 5 days (75 μg per injection) via a porous indwelling catheter underlying the incision, EGF-treated incisions were approximately 35% stronger than saline-treated incisions at 7 days (p<0.01, t-test). Furthermore, a single treatment with a total of 3 μg of EGF encapsulated in multilamellar liposomes (ML-EGF) caused approximately 300% (p<0.05, t-test) increase in tensile strength at 10 and 14 days post-incision compared to blank liposomes or untreated incisions (Figure 5). Histology of 7 day incisions treated with EGF in liposomes showed hypertrophy of the overlying epidermis and substantially more fibroblast-like cells in the area of the incision tract compared to control incisions. Electron microscopy of 14 day incisions treated with EGF showed many active fibroblasts present with extensive

-6-

rough endoplastic reticulum and more new collagen present than control incisions. Specimens at 28 days treated with EGF showed large numbers of very active fibroblasts, extensive well organized collagen with sparse ground substance giving the overall appearance of a very strong, mature scar, while control specimens retained large amounts of edematous and spongy ground substance indicating earlier stages of wound repair.

These results suggested a direct link between the residency time of EGF in the incision and the effectiveness of EGF in stimulating healing of incisions. To measure the residency time of EGF in incisions, EGF containing a trace of ^{125}I-EGF was formulated in saline, hyaluronic acid, multilamellar or single lamellar liposomes and placed in tracts of incisions. The percentage of EGF remaining in the region surrounding the incisions was measured daily by scintillation counting (Figure 6). After one day, only 20% of the EGF formulated in saline or hyaluronic acid remained in the region surrounding the incision. EGF encapsulated in single lamellar lipsomes also was rapidly lost from the incision area, but EGF encapsulated in multilamellar liposomes was substantially retarded in the area of the incision with 40% still retained at three days.

Although EGF required special formulations which prolonged its release in incisions, a single application of TGF-β in soluble collagen produced a significant increase in tensile strength at 10 days post-surgery during the early phase of healing (Figure 7). At a later stage of healing (14 days), tensile strengths were equal for TGF-β and control incisions.

Tympanic Membrane Perforations. As part of an initial study on the potential usefulness of EGF in stimulating healing of TM perforations, we determined if TM is a target tissue for EGF. Using ^{125}I-EGF, we performed binding studies on porcine TMs and found that TMs expressed substantial levels of specific, high affinity receptors for EGF (Figure 8). Using autoradiography of ^{125}I-EGF binding to intact TMs, we localized EGF receptors to all three cell layers of the TM with the highest level of receptors found in the stratified epithelial layer. Treatment of TM perforations in cats with EGF in saline or hydroxypropylmethyl cellulose vehicles demonstrated that EGF induced extensive hyperplasia in the stromal and epithelial layers. EGF treatment also caused faster closure of TM perforations than in contralateral TMs treated with vehicle in 3 of 4 cats (Figure 9).

DISCUSSION AND CONCLUSIONS

The results of the experiments conducted during the two years of this contract clearly demonstrate that addition of peptide growth factors to injuries can accelerate healing in a variety of wounds. Specifically, biosynthetic human EGF increased the rate of epidermal regeneration of mid-dermal injuries in both animal models and in a clinical trial. EGF and TGF-β both increased tensile strength of surgical incisions in rats during the early phase of healing, and EGF increased the rate of healing of TM perforations in cats.

During the course of these experiments other important aspects were recognized regarding the use of growth factors to stimulate wound healing. One of the most important concepts was that EGF must be applied in vehicles that allow it to remain in contact with target cells for extended periods. Three previous reports (3,4,5) all failed to detect any stimulation of epidermal regeneration by EGF probably because the vehicle and manner of application of EGF did not provide an adequate period of exposure (6 to 12 hours). Tissue

culture experiments had shown previously that target cells in vitro required extended periods of exposure to EGF for stimulation of mitosis (7,8). Thus, it is also important to have a basic understanding of the biochemical mechanism of action of growth factors so that experiments can be designed which will reliably test the effects of growth factors on wound healing.

Additional epidermal regeneration experiments demonstrated that not all growth factors act to stimulate healing. TGF-β in particular may inhibit epidermal regeneration, and IGF-I and bFGF may be relative ineffective in comparison to EGF. Additional experimentation needs to be conducted to unequivocally establish the effects of these new growth factors. For example, different doses and different vehicles should be evaluated and combinations of growth factors should be evalua:ed more extensively.

If topical application of TGF-β can be shown to consistently and substantially retard epidermal regeneration, it suggests that mid-dermal injuries that heal slowly may be a result of excess local production of TGF-β. New therapeutic concepts could be based on interfering with the actions of TGF-β by antibodies that neutralize TGF-β or by antibodies that block binding of TGF-β to its receptor with out activating the receptor.

Results of the clinical trial clearly demonstrate that healing of mid-dermal injuries is accelerated by topical treatment with EGF. It is important to recognize that the clinical trial utilized normal, noncompromised donor site injuries. Thus, EGF treatment accelerated healing in normal tissue. Preliminary results with four patients with chronic, nonhealing ulcers treated with EGF are very encouraging. All four ulcers healed with EGF treatment in less than three weeks. A controlled trial with EGF in diabetic ulcers is just beginning at the University of Louisville and at other universities.

Compared to mid-dermal injuries, the use of growth factors to accelerate healing of surgical incisions has an added technical difficulty since only a single, local application of the growth factor can be made at the time the incisions is closed. Our results demonstrated that EGF was not retained in incisions for sufficient periods to increase tensile strength when it was formulated in simple vehicles such as saline or hyaluronic acid. However, when EGF was formulated in liposomes, EGF was retained in incisions for an extended period which induced a significant increase in tensile strength during the early phase of healing. In contrast, both we and Mustoe et al. (15) found that treatment of incisions with a single application of TGF-β in a soluble collagen vehicle also significantly increased tensile strength of paired incisions. The ability to use a single application of TGF-β in a simple vehicle, i.e. soluble collagen, suggests that TGF-β may be much easier to adapt to clinical use than EGF because of the ability to use a much simpler vehicle (soluble collagen verses liposomes). Also, TGF-β may not require prolonged, continuous exposure to cells to induce a biological response important for incisional healing.

TGF-β has been reported to both stimulate and inhibit cell mitosis leading to the concept that TGF-β is a bifunctional regulator of cell growth in vitro (13,14). Our in vivo results with TGF-β also show an apparent bifunctional response with stimulation of incisional healing but an inhibition of epidermal regeneration on donor sites. Obviously, there is a need for more research to fully understand how TGF-β regulates healing in these two different injuries. It is possible that the major mechanism of action of TGF-β in incisions is indirect with TGF-β acting as a chemoattractant for macrophages which influence healing by releasing peptide growth factors in the area of an incision. In epidermal regeneration, TGF-β may act by directly inhibiting keratinocyte mitosis in vivo as has been reported for human keratinocytes in vitro (13).

TM has not been investigated previously for interaction with growth factors. Our data clearly demonstrated that TM is a target tissue for EGF, and that topical treatment of TM perforations with EGF induced substantial hyperplasia of the epithelial and stromal layers and produced more rapid closure of the perforations. More thorough research needs to be conducted on the ability of EGF or other growth factors to speed healing of TM perforations and allow faster rehabilitation of hearing.

In summary, the concept of using exogenous growth factors to accelerate healing of mid-dermal injuries, incisions and TM perforations continues to be viable. Data from the first clinical trial of EGF in epidermal regeneration supports the data gathered from the pig model and validates the hypothesis for epidermal regeneration. The dramatic results of the initial cases of chronic ulcers treated with EGF justify a double-blinded cross-over clinical trial evaluating the effect of EGF treatment on chronic ulcers. Results of EGF and TGF-β treatment of incisions and TM perforations in animal models holds promise of clinical usefulness once adequate preclinical studies are completed.

What important questions are left for future investigation? One very important area that has not been investigated is to determine what peptide growth factors are produced in a wound during the course of normal healing. We are investigating this question by measuring mRNA levels for 9 different growth factors in specimens of regenerating mid-dermal injuries and incisions. We are using molecular hybridization techniques with cDNA probes specific for EGF, TGF-α, TGF-β, bFGF, aFGF, IGF-I, IGF-II, PDGF, and IL-I. We are also investigating whether EGF receptor levels change during healing of these wounds. Once we have determined the roles of these growth factors in normal wound healing, we will determine if they are altered in conditions of impaired wound healing such as diabetes or steroid treatment.

REFERENCES

1. G Carpenter and S Cohen. Human epidermal growth factor and the proliferation of human fibroblasts. Cell Physiol 88:227, 1975.

2. J G Rheinwald and H Green. Epidermal growth factor and the multiplication of cultured human epidermal keratinocytes. Nature (London) 265:421, 1977.

3. M W Greaves. Lack of effect of topically applied epidermal growth factor (EGF) on epidermal growth in man in vivo. Clin Exper Dermatol 5:101, 1980.

4. G Arturson. Epidermal growth factor in the healing of corneal wounds, epidermal wounds and partial-thickness scalds. Scand J Plast Reconstr Surg 18:33, 1984.

5. J W Thorton, C A Hess, V Cassingham, R H Barlettt. Epidermal growth factor in the healing of second degree burns: a controlled animal study. Burns 8:156, 1981.

6. J D Franklin and J B. Lynch. Effects of topical applications of epidermal growth factor on wound healing. Plast Reconstr Surg 64:766, 1979

7. A Aharonov, R M Pruss, H R Herschman. Epidermal Growth Factor, Relationship between receptor regulation and mitogenesis in 3T3 cells. J Biol Chem 253:3970, 1978.

8. H T Haigler, G Carpenter. Production and characterization of antibody blocking epidermal growth factor:receptor interactions Biochim Biophys Acta 598:314, 1980.

9. A Buckley, J M Davidson, C D Kamerath, T B Wolt, S C Woodward. Sustained release of epidermal growth factor accelerates wound repair. Proc Natl Acad Sci USA 82:7340, 1985.

10. M Laato, J. Niinikoski, B Gerdin, and L Lebel. Stimulation of wound healing by epidermal growth factor. Ann Surg 203:379, 1986.

11. G R Grotendorst, G R Martin, D Pencev, J Sodek, and A K Harvey. Stimulation of granulation tissue formation by platelet-derived growth factor in normal and diabetic rats. J Clin Invest 76:2323, 1985.

12. G L Brown, L Curtsinger, III, J R Brightwell, D M Ackerman, G R Tobin, H C Polk Jr, C George-Nascimento, P Valenzuela, G S Schultz. Enhancement of epidermal regeneration by biosynthetic growth factor. J Exp Med 163:1319, 1986.

13. A B Roberts, M A Anzano, L M Wakefield, N S Roche, D F Stern, M B Sporn. Type β Transforming growth factor: A bifunctional regulator of cellular growth. Proc Natl Acad Sci USA 82:119, 1985.

14. H L Moses, R F Tucker, E B Leof, R J Coffey Jr, J Halper, G D Shipley. Type-B transforming growth factor is a growth stimulator and a growth inhibitor. Cancer Cells, Growth Factors and Transformation, Cold Spring Harbor, pp. 65-71, 1985.

15. T A Mustoe, G F Pierce, A Thomason, P Gramates, M B Sporn, T F Deuel.
     Accelerated healing of incisional wounds in rats induced by transforming
     growth factor-β. Science 237:1333-1336, 1987.

16. F Szoka Jr and D Papahadjopoulos. Procedure for preparation of liposomes
     with large internal aqueous space and high capacity by reverse-phase
     evaporation. Proc Natl Acad Sci USA 75:4198, 1978.

TABLE 2

Clinical Trial of hEGF on Paired Donor Sites. Twelve patients received paired
donor sites 5 x 15 cm created with a Padgett dermatome set at a depth of 12/1000
inch. Wounds were treated twice daily with 0.5 ml/cm^2 of Silvadene containing
10 μg hEGF/ml or with Silvadene alone. Wounds were photographed daily and
prints were analyzed by masked evaluators for epidermal regeneration using
computerized planimetry.

treatment	days to complete healing (mean)
Silvadene	10.75
Silvadene + EGF	8.10

TABLE 1

Effect of hEGF and Vehicle on Healing of Partial-thickness Burns

Treatment	Percentage of burn area healed
hEGF	93 ± 6
Control	36 ± 18
Untreated	17 ± 11

$p < 0.01$ using one-way analysis of variance and Tukev's Honest Statistical
Difference (HSD) test. Quantitative planimetry measurements were per-
formed on photographs of partial-thickness burns that were treated for 7
d with hEGF in Silvadene (10 μg/ml). Silvadene alone (control) or un-
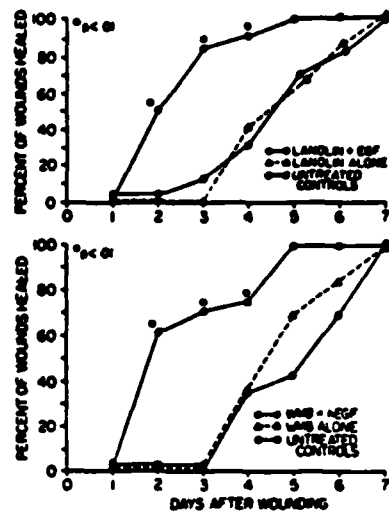treated. Values are mean ± SD for eight wounds.

FIGURE 1. Effects of hEGF and vehicles on healing of split-thickness wounds. Each point represents the data from 16 wounds treated with hEGF (10 µg/ml) in the indicated vehicles, with vehicles alone, or with no treatment. Values were compared for statistical significance by χ^2 analysis. WMB, water-miscible base (Silvadene).
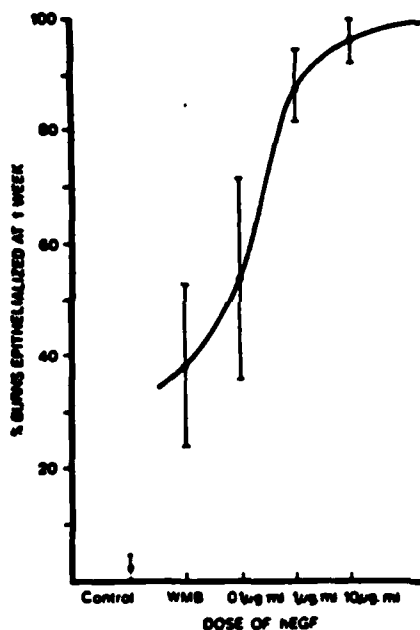

FIGURE 2. Response of partial-thickness burns to varying doses of hEGF. Partial-thickness burns (3 × 3 cm) were treated for 7 d with Silvadene alone, Silvadene containing hEGF (10 µg/ml, 1 µg/ml, 0.1 µg/ml), or were not treated. The percentage of original burn area that had healed was measured by quantitative planimetry of photographs. Each data point is the mean ± SD for four burns. WMB, water miscible base (Silvadene).
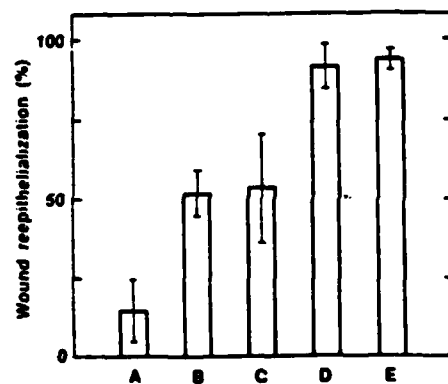
Fig. 3 Relative abilities of various treatment regimens to facilitate epithelial wound healing. The growth factors were all tested at 0.1 µg/ml. This concentration has previously been shown to be suboptimal for EGF (2). Conditions are (A) untreated; (B) Silvadene alone; (C) Silvadene and EGF; (D) Silvadene and TGF; (E) Silvadene and VGF. Results were scored at 9 or 10 days and are the average of two or more experiments with different test animals. Results are mean ± standard deviation.

12

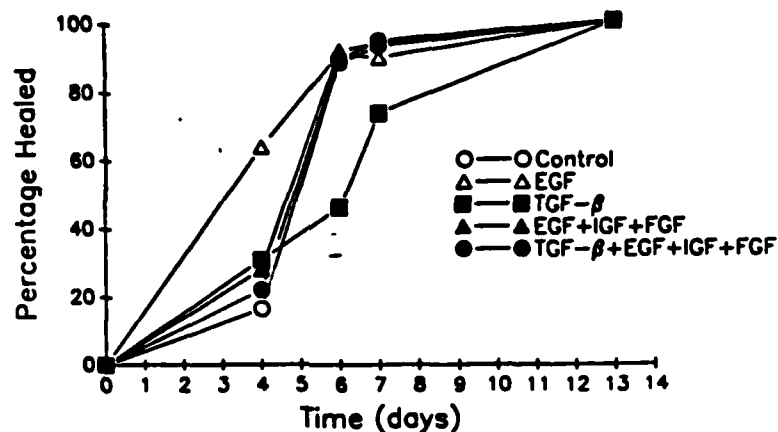## Donor Site Healing With Growth Factors in Release Dressings

Figure 4. Donor Site Healing with Growth Factors and Release Dressings. Paired donor sites were made on the backs of pigs with a dermatome then treated topically once daily with an occlusive release dressing soaked with saline vehicle both with and without test growth factors. At the indicated times after injury dressings were removed, donor sites were photographed, and healing evaluated by planimetry of the photographs. Values are the mean of 3 to 6 donor sites. Factors were tested at 10 ug per ml.
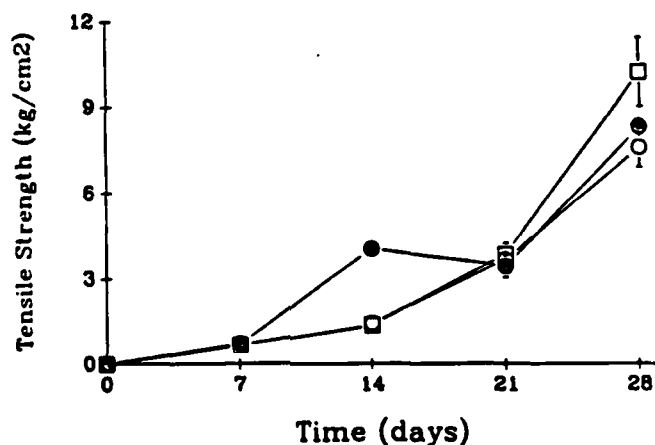
Figure 5. Tensile strength of incisions treated with EGF-liposomes. Single incisions 5 cm long were made to the panniculus carnosum in the dorsal midline at the caudal portion of rats and EGF (3 ug) encapsulated in multilamellar liposomes or blank liposomes placed in the tract of the incision which was then closed with five interrupted sutures. Incisions were excised at weekly intervals and tested for tensile strength. Values represented are the mean of seven incisions and T-test was used for statistical comparisons.
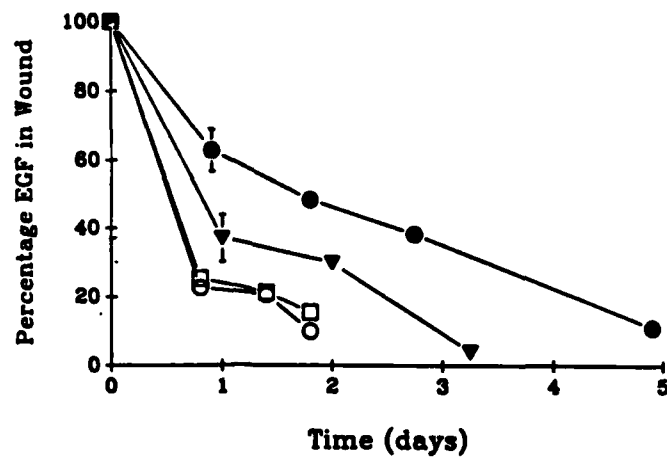
FIG 6. RETENTION OF EGF IN INCISIONS. Forty-eight adult male Sprague-Dawley rats were divided into four equal groups. One of four formulations containing 125I-EGF and unlabeled insulin as carrier was then placed in the base of the incisions: multilamellar liposomes (●), single lamellar liposomes (▼), hyaluronic acid (□), saline (O). Values are the mean and standard deviation of three incisions.
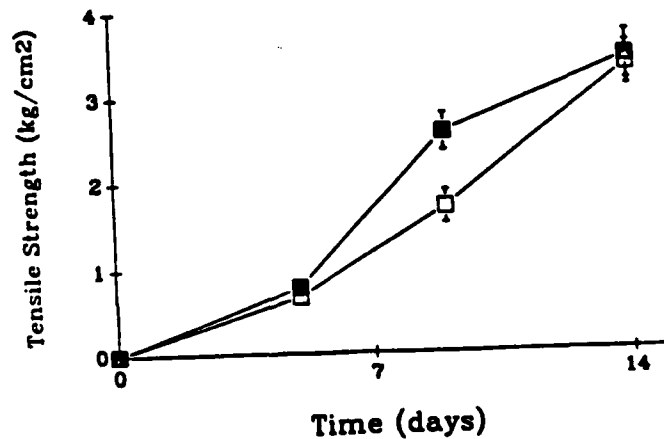

Figure 7. Tensile Strength of Incisions Treated with TGF-β. Paired linear incisions were made through the dorsal skin of rats, soluble collagen with or without 2 ug of TGF-β were added at the base of the incision which was closed with 5 interrupted sutures and at the indicated times, incisions were tested for tensile strength. Values are the mean and standard error for 9 measurements.
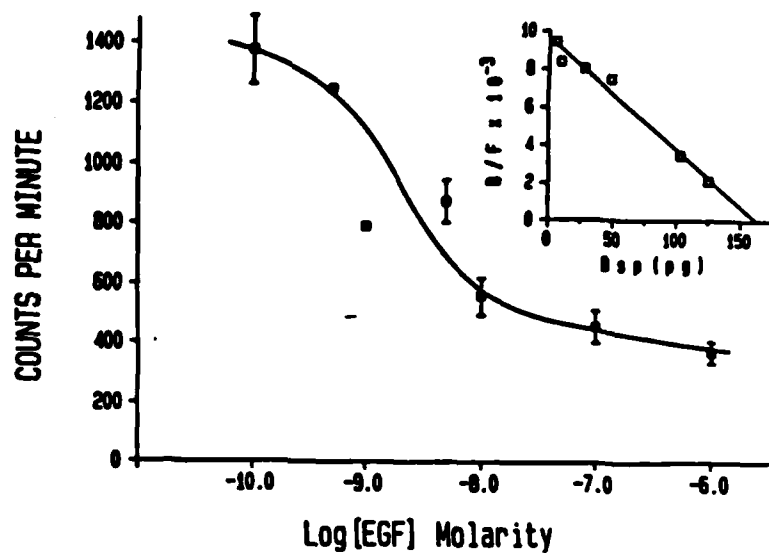
Figure 8. Characterization of EGF Binding to TM. Intact porcine TMs were incubated in CDM at 37°C for 2 hours containing ^{125}I-EGF (100 pM) and increasing concentrations of unlabeled EGF (10 pM to 1 μM). Specific binding was transformed by method of Scatchard (insert).
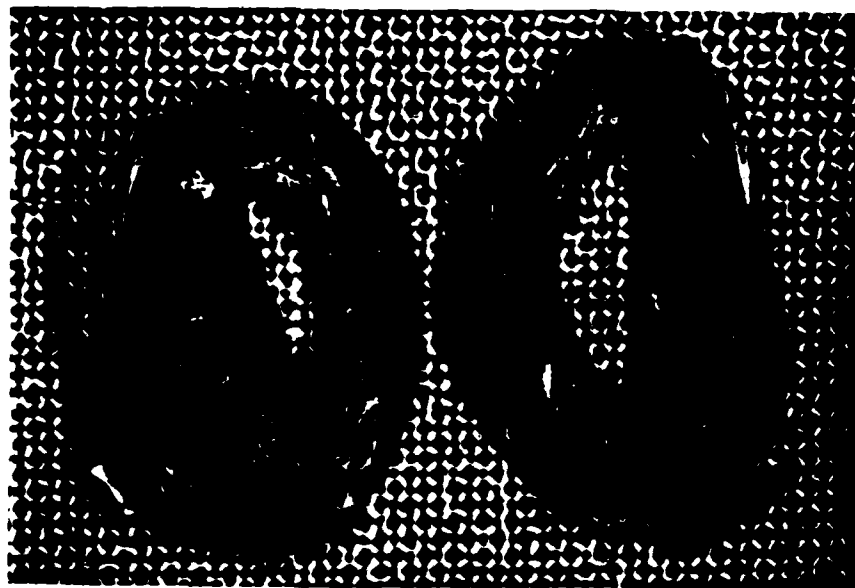

Figure 9. Gross Morphology of Perforated Cat TMs Treated with EGF Impregnated Gel-foam. Total perforations of cat TMs were dosed once with Gel-foam impregnated with EGF (50 μg, left) or saline (right) at the time of surgery then fixed in situ six days later.

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